Isolation and Detection of Dialkyl Phthalates from Pork

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Thin-layer chromatography (TLC), Fourier transform infrared spectroscopy (FTIR), and gas chromatography–mass spectrometry (GC–MS) indicate that lipid extracts from pork fat taken from commercial cuts of meat contain, as minor constituents, dialkyl phthalates (DAP). GC–MS revealed further that the most abundant phthalate component was a dioctyl ester. This class of compounds migrates on TLC plates with the neutral lipids, but when chromatographed on a silicic acid column these species appear in the glycolipid fraction. Examination of extracts from the plastic with which the meat was wrapped showed no aromatic esters. Thus, these substances either were accidentally introduced during processing or were already present in the live animal.

Phthalates are widely used by chemical manufacturers as plasticizers. The most commonly used of these is bis-(2-ethylhexyl) phthalate, commercially known as dioctyl phthalate or DOP (Bemis et al., 1982). These esters have been detected in a variety of animal tissues, including eggs layed by hens to which DOP was directly administered (Ishida et al., 1981) and in samples of bovine milk (Cerbulis and Ard, 1967). In some instances phthalate esters may be introduced during food processing and handling due to contact with specific plastic products; in other cases, the phthalates may have already been present in the animal prior to slaughter.

During a study of the composition of hydrocarbons and sphingolipid bases from pork, a minor, previously unreported constituent was found in the lipid fraction isolated from the fatty tissues. Thin-layer chromatography (TLC), Fourier transform infrared (FTIR) spectroscopy, and gas chromatography–mass spectrometry (GC–MS) indicate that this newly detected material consists principally of a mixture of dialkyl phthalates (DAP). The most abundant component observed by GC–MS was a dioctyl phthalate ester.

MATERIAL AND METHODS

Solvents and Column Packing Materials. Nanograde solvents were used throughout this study. Evaporation of these materials showed no traces of phthalates or other organic contaminants detectable by TLC. Silicic acid was washed with ether and chloroform–methanol (2:1) prior to use.

Pork. To obtain a representative sample of pork fat, pork products were bought from different supermarkets. The following principal cuts of pork were used: fresh ham, fresh picknic shoulder, and feet. After the plastic wrap was removed from the pork, all further contact with plastics was scrupulously avoided. The tissue was chopped in a meat chopper and then thoroughly mixed to make a homogeneous sample. Prior to handling the pork products, all containers and the chopper were washed with chloroform.

Lipid Extraction. The lipids were obtained by four subsequent extractions of the homogeneous pork sample described above with chloroform–methanol (2:1, v/v), as reported previously (Cerbulis, 1967) with a ratio of 20 mL of solvent/1 g of sample. The extracts were evaporated to dryness; then, the residue was taken up with chloroform–methanol (2:1) and washed with 0.2 volume of water. The $CHCl_3$ layer was evaporated to dryness and analyzed by TLC and column chromatography.

TLC. Sybron/Brinkmann Sil G-25 precoated TLC plates were used. Developing solvents were petroleum ether–diethyl ether–acetic acid (90:10:1, v/v/v) for neutral lipids and chlorofrom–methanol–water (65:25:4, v/v/v) for polar lipids (glycolipids, phospholipids). Reagent-grade ferric chloride–sulfuric acid was used to visualize the separated lipids (Cerbulis et al., 1984).

Silicic Acid Column Separation of Pork Fat. Initial separation of the components in the lipid extract was carried out on Unisil columns (2.5 × 25 cm) as described previously (Cerbulis et al., 1983): fraction A, eluted with chloroform, contained neutral lipids; fraction B, eluted with acetone, contained glycolipids; fraction C, eluted with methanol, contained phosphatidyl lipids and sphingomyelin. Each fraction was analyzed by TLC using both solvent systems. Fraction B was subjected to preparative TLC using chloroform–methanol–water solvent described above. The TLC spot subsequently identified as DAP (see Results) was removed and redissolved in chloroform to obtain samples for examination by FTIR spectroscopy and GC–MS.

Extraction of Plastic (Wrapping Material). The plastic with which the pork was wrapped was also analyzed

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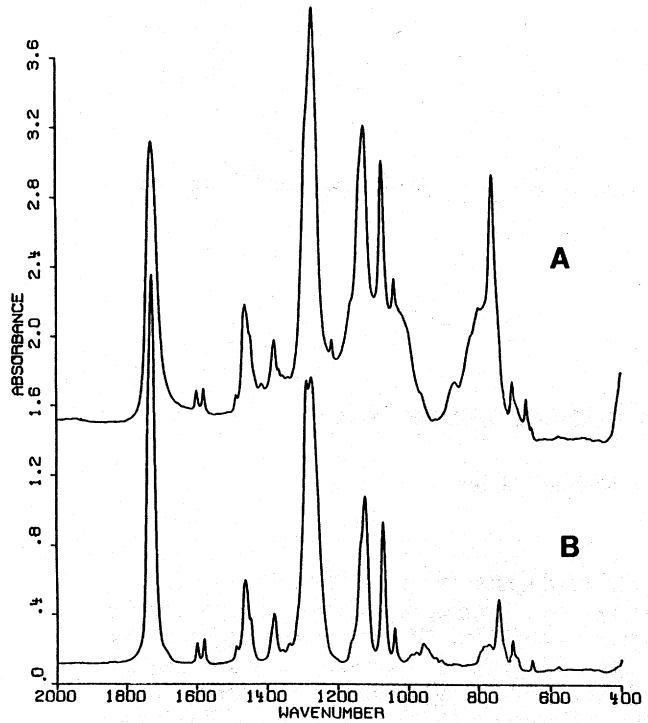

Figure 1. FTIR spectra: (A) dialkyl phthalate mixture isolated from pork fat, 2000–400 cm^{-1} broad, underlying spectral features near 1000 and 800 cm^{-1} likely due to traces of solid particulates from the coating of the TLC plate; (B) pure dioctyl phthalate, 2000–400 cm^{-1}.

to check whether this might be a potential source of the DAP. (Identical, unused plastic wrapping material was also obtained from the same supermarkets and subjected to an identical analysis.) The plastic wrap was cut into small pieces; 1 g was then extracted with 20 mL of chloroform–methanol (2:1) in exactly the same manner as was done with the pork. This extract was then examined by TLC and FTIR spectroscopy.

Extraction of the Coating of a TLC Plate. The coating of an unused TLC plate was scraped off and extracted with benzene and methylene chloride. The extracts were combined, evaporated, and examined for organic contaminants by FTIR spectroscopy.

Infrared Spectra. All infrared spectra were obtained on a Nicolet 7199 FTIR spectrophotometer (Madison, WI) equipped with a KBr beamsplitter and a HgCdTe detector. The spectra were obtained at 2-cm^{-1} resolution with one level of zero-filling of the interferogram prior to apodization by the Happ–Genzel function. Chloroform solutions of samples analyzed by TLC were placed on KBr plates, and the solvent was removed by evaporation under a stream of dry-N_2 gas. The greasy residue was then pressed between the plates and the FTIR spectrum obtained. The spectrum of pure, liquid DOP (reagent grade, Aldrich Chemical Co.) was also obtained as a thin film between plates.

GC–MS. Mass spectra were obtained on a Hewlett–Packard HP-5995C (Palo Alto, CA) GC–mass spectrometer with a 12.5 m × 0.2 mm cross-linked dimethyl silicone fused-silica capillary column programmed 90–250 °C at 16 °C/min and with a He flow rate of 30 cm/s. The mass spectrometer was timed by using the autotime program and operated at default parameter values.

RESULTS

An unknown group of compounds was isolated with the polar glycolipid fraction of pork fat by the silicic acid column separation, but on a TLC plate this material migrated as a neutral lipid. The unknown was isolated from the glycolipid fraction by preparatory TLC; when $FeCl_3$ reagent was added to the TLC plate, the substance gave a characteristic blue color reaction identical with that observed with pure DOP. By contrast, other neutral lipid components gave gray reactions, distinctly different from the blue seen for DOP and the unknown. The R_f values, using the 90:10:1 solvent, for both the standard DOP and the uncharacterized compound from pork, were 0.72, indicating that the compound was similar in structure to DOP. On the other hand, extracts from the plastic wrapping material showed several blue spots, one with R_f 0.55 and the others with R_f 0.00–0.20, as well as two small gray spots with R_f 0.72 and 0.90. So analysis by TLC implies that these extracts from the plastic contained neither DOP nor the unknown. Thus, the pork fat was not contaminated by the wrapping material, nor by interference were the solvents the source of this unknown substance.

Table I. Observed Frequencies (cm^{-1}) of the Principal Bands in the Infrared Spectra of Dioctyl Phthalate (DOP), Dialkyl Phthalate from Pork (DAP), and a Mixture of Esters Extracted from the Plastic Food Wrap

DOP	DAP	esters
3071 vw	3069 vw	
	3021 vw	
2960 m	2961 s	2958 s
2931 m	2927 ms	2929 ms
2874 mw	2872 m	2873 m
2861 mw	2855 m	2859 m
		1737 ms
1728 s	1728 ms	
1600 vw	1600 vw	
1581 vw	1581 vw	
1488 vw	1488 vw	
1463 mw	1463 mw	1463 mw
1449 w, sh	1449 w, sh	
	1415 vw	1419 w
1381 w	1380 w	1379 w
		1365 w
		1310 vw
1288 ms		1283 w
1274 ms	1269 vs	
		1242 mw
	1216 w	
		1173 m
		1143 mw
1123 m	1124 ms	
1073 mw	1074 m	1077 w
1040 vw	1040 mw	
960 vw		
	865 w	
	797 mw	
780 vw		
	760 m	766 vw
743 mw		
705 vw	705 vw	
	668 vw	
652 vw		

Figure 1 shows the FTIR spectra of the compound from pork (A) and the pure DOP (B). Although not identical, the similarities in the two spectra are sufficient to identify the unknown material as consisting of one or more dialkyl phthalates (DAP), thus corroborating the TLC data. In particular, the ester carbonyl (C=O) stretching frequency is the same, 1728 cm^{-1} (Figure 1; Table I) , and both also exhibit bands characteristic of ortho-disubstituted benzene rings at ca. 3070 and 3021 cm^{-1} (Table I) and at 1600, 1580, 1488, and 1449 cm^{-1} (Figure 1; Table I) (Colthup et al., 1975). Because the ratio of the peak intensities (in absorbance units) of the CH deformation bands at 1460 and 1380 cm^{-1} to those of the C=O stretching band at 1728 cm^{-1} is somewhat greater for the DAP mixture than for

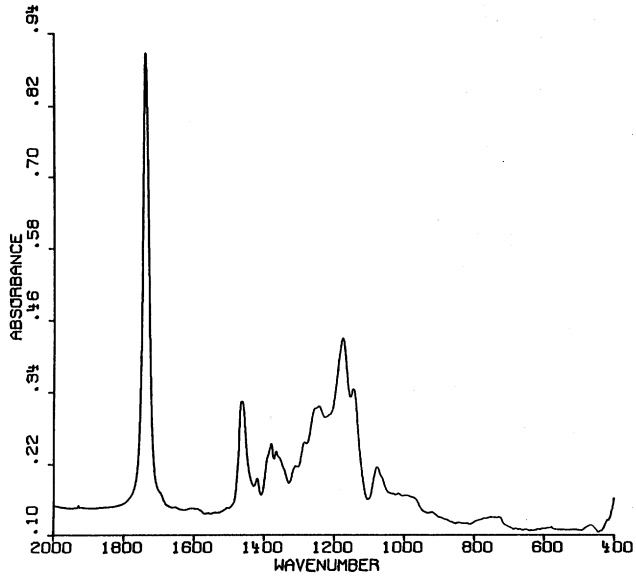

Figure 2. FTIR spectrum of ester isolated from plastic wrapping material, 2000–400 cm^{-1}.

the pure DOP (0.39 vs. 0.22), one is led to conclude that the mixture of phthalate esters isolated from pork has a higher proportion of alkyl carbons to aromatic carbons than does pure DOP. In addition, the absence of a band near 722 cm^{-1} (due to the rocking motions of methylene chains having four or more carbon atoms) implies that, as in DOP, the alkyl groups of the DAP are probably branched.

The chromatogram obtained with the GC–MS exhibited three components, each of whose spectra had base peaks at m/z 149 characteristic of phthalates. A computer search of the MS spectral library identified the major component peak as diisooctyl phthalate and the two smaller components as dibutyl phthalate and diisodecyl phthalate. The identify of the major component as a dioctyl phthalate was confirmed by running pure DOP as a reference.

The chromatogram of the TLC extract also revealed two other bands of unknown structure. These components appear to be structurely related to one another but are clearly not phthalates. Because these two peaks overlapped the phthalate components in the chromatogram, they were subtracted out before any library searches were made to identify the phthalate components.

Figure 2 shows the FTIR spectrum of the extract from the plastic wrap. TLC indicates that this extract is a mixture of at least five different species. Nonetheless, whatever the identify of these components from the plastic, FTIR clearly shows that this mixture consists of a very different sort of ester from that of the phthalates. In this case, the observed carbonyl stretching frequency is nearly 10 cm^{-1} higher (Figure 2; Table I) than found for the phthalates. In addition, the absence of the characteristic bands near 3050, 1600, and between 700 and 800 cm^{-1} (Colthup, et al., 1975) unambiguously demonstrates that this mixture consists largely of nonaromatic species. Also, the spectrum in Figure 2 shows no strong band near 1270 cm^{-1}, as do both DOP and DAP. These results corroborate the conclusions reached by the TLC analysis: the plastic wrap seems not to be the source of the phthalate esters found in the pork. An FTIR spectrum of an extract from the TLC coating of a blank plate shows traces of an organic binder, but spectroscopically this substance clearly bears little resemblance to a phthalate ester either. Therefore, one must conclude that the DAP was already present in the pork itself prior to the time it was wrapped.

Various phenolic acids have been reported to be present in the urine of pig, cow, and dog and in pig's slurry (Garraway and Ramirez, 1982; Spoelstra, 1977). Nevertheless, the ultimate source of the phthalate esters found in pork by this study remains an open question. Perhaps the DAP was introduced accidentally during butchering or processing; alternatively, it could possibly have already been present in the live animal. In any case, the potential toxicity of such species to both animals and humans suggests the need for further investigation into the source of such materials in the food chain.

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